

Delayed fatty acid uptake by a fatty acid secreting yeast strain with modified peroxisomal metabolism

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Introduction

Biodiesel, in the form of fatty acid esters, produced by oleaginous microorganisms, could represent an attractive alternative of renewable liquid biofuels.

Lipid metabolism has been studied extensively in *S. cerevisiae* and all genes encoding enzymes directly involved in lipid synthesis are known. In this model yeast, exogenous long-chain fatty acids are activated to coenzyme A derivatives prior to metabolic utilization by the fatty acyl-CoA synthetases Faa1p and Faa4p. Free fatty acids are secreted from an *FAA1,4* double mutant (1), which we use as a basis for metabolic engineering of this property. However, secreted fatty acids disappear late in the growth phase, presumably metabolized by the cells (1).

In *S. cerevisiae* the acyl-CoA oxidase ScPox1p catalyzes the first metabolic step of fatty acid beta-oxidation, and null mutants are unable to grow on fatty acids as sole carbon source (2). ScPox1p is the ortholog of the human acyl-CoA oxidase 1, and its expression is strongly induced by fatty acids. A triple knock-out mutant *faa1/4-Δ1 pox1-Δ1* studied in this work shows delayed uptake of the secreted fatty acids.

Methods

Genetic modifications

In order to create the *FAA1,4* double deletion, we used a new system for clean gene deletions called Delitto Perfetto (3). The system was modified to include a long PCR product covering the deletion site made through fusion PCR. This proved to be a very efficient system, leaving no trace of foreign DNA in the strain. The CEN.PK 115-5d *faa1/4-Δ1* deletion mutant was used as a host for the deletion of *POX1* using the *loxP*–*kanMX*–*loxP* gene disruption cassette (4).

Measurement of fatty acid concentration in culture supernatant by optical density

Two milliliters of culture were collected at time points indicated and centrifuged 5 min at 14000 rpm. The optical density of the supernatant was measured in a Thermo Spectronic Genesys 21 spectrophotometer at 600nm.

Cell growth and Lipid analytical methods

Yeast cultures were grown at 30°C in YPD medium (1% yeast extract, 2% peptone and 2% dextrose).

At the time points indicated, 2mL of the culture were collected and the extracellular fraction was extracted with 2mL of n-hexane, after acidification, using pentadecanoic acid as internal standard. The lipids extract was dried under a stream of nitrogen and methylated in 500uL of BF_3 /methanol, 30 min at 90°C. The mixture was then extracted with 500uL of n-hexane and the fatty acid composition of the supernatant was determined by Gas Chromatography (GC) of fatty acid methyl esters (FAMES). FAMES were identified by comparing their retention times with those of standards.

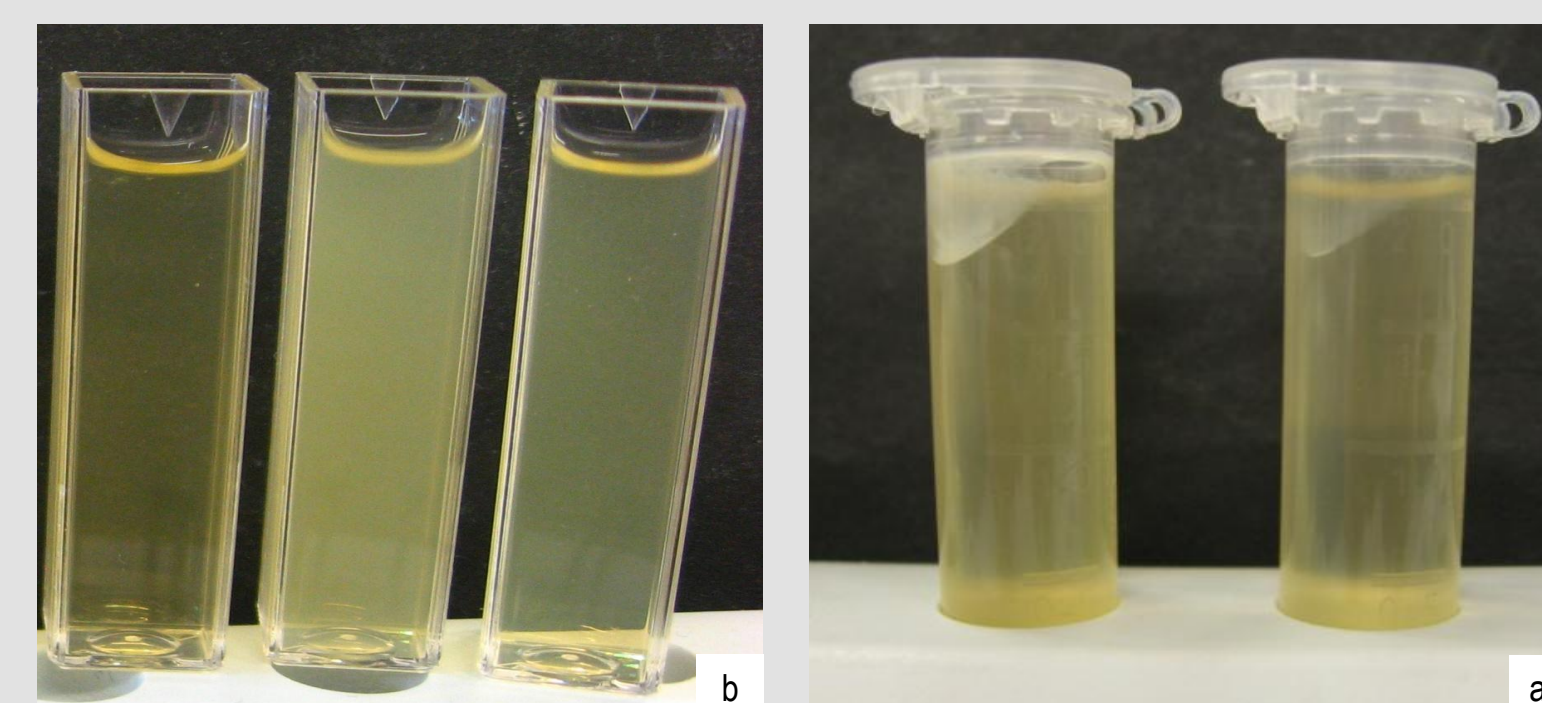


Fig.1: Example of the visual aspect of the extracellular media of fatty acid secreting strains after 30 hours of growth. a) Supernatant after centrifuging 5 min, 14000 rpm. b) Different optical densities measurable with a spectrophotometer.

Results

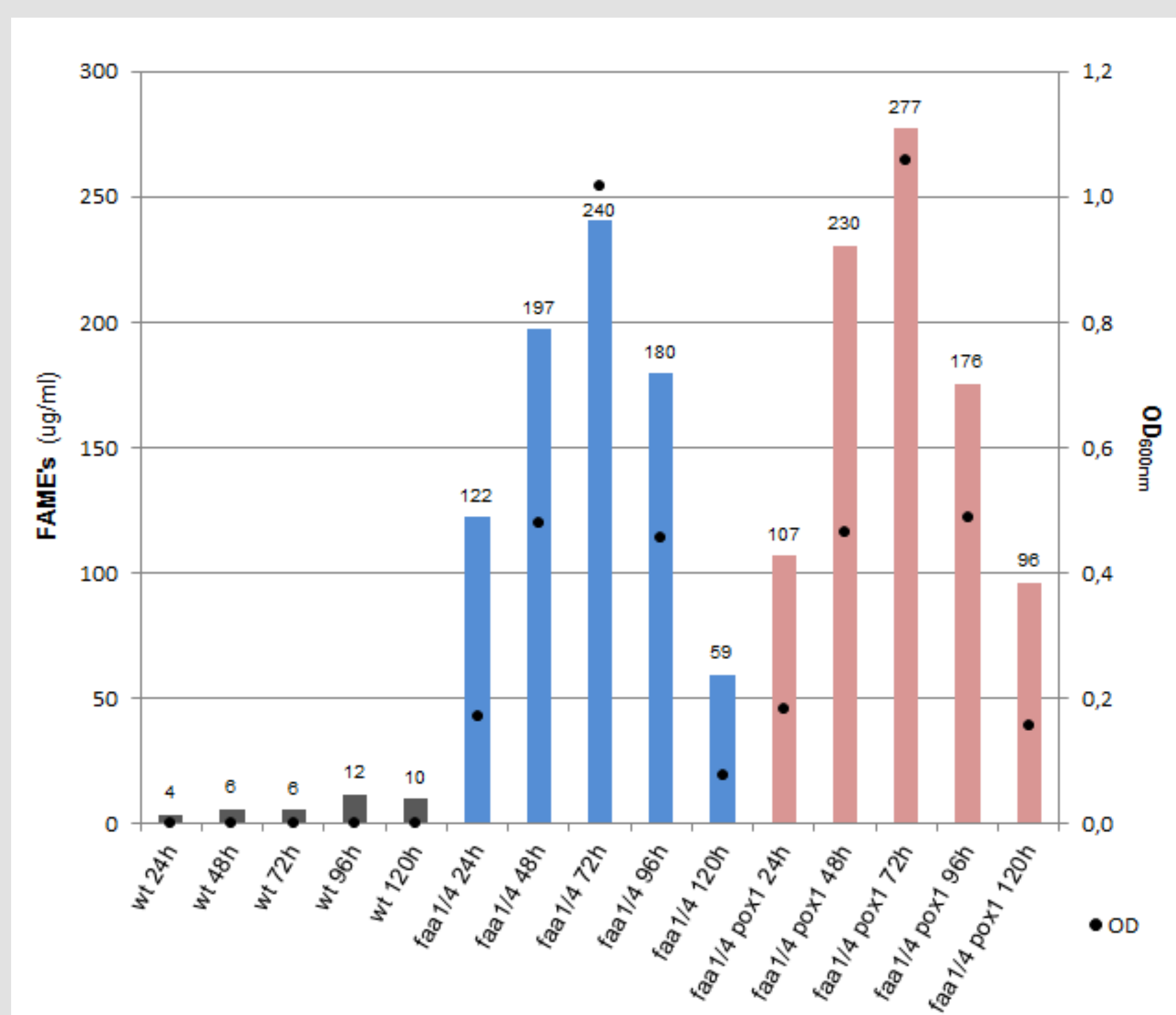


Fig. 2: Analysis of the FAMES composition of the culture media by GC. Extracellular accumulation of free fatty acids in the culture medium was determined at the time points indicated. Also a fatty acid re-import phenotype was observed, in agreement with results already obtained by Scharniewski *et al.* 2008, indicating fatty acid uptake during stationary phase. The triple knock-out mutant *faa1/4-Δ1 pox1-Δ1* shows delayed uptake when compared with the *faa1/4-Δ1* mutant.

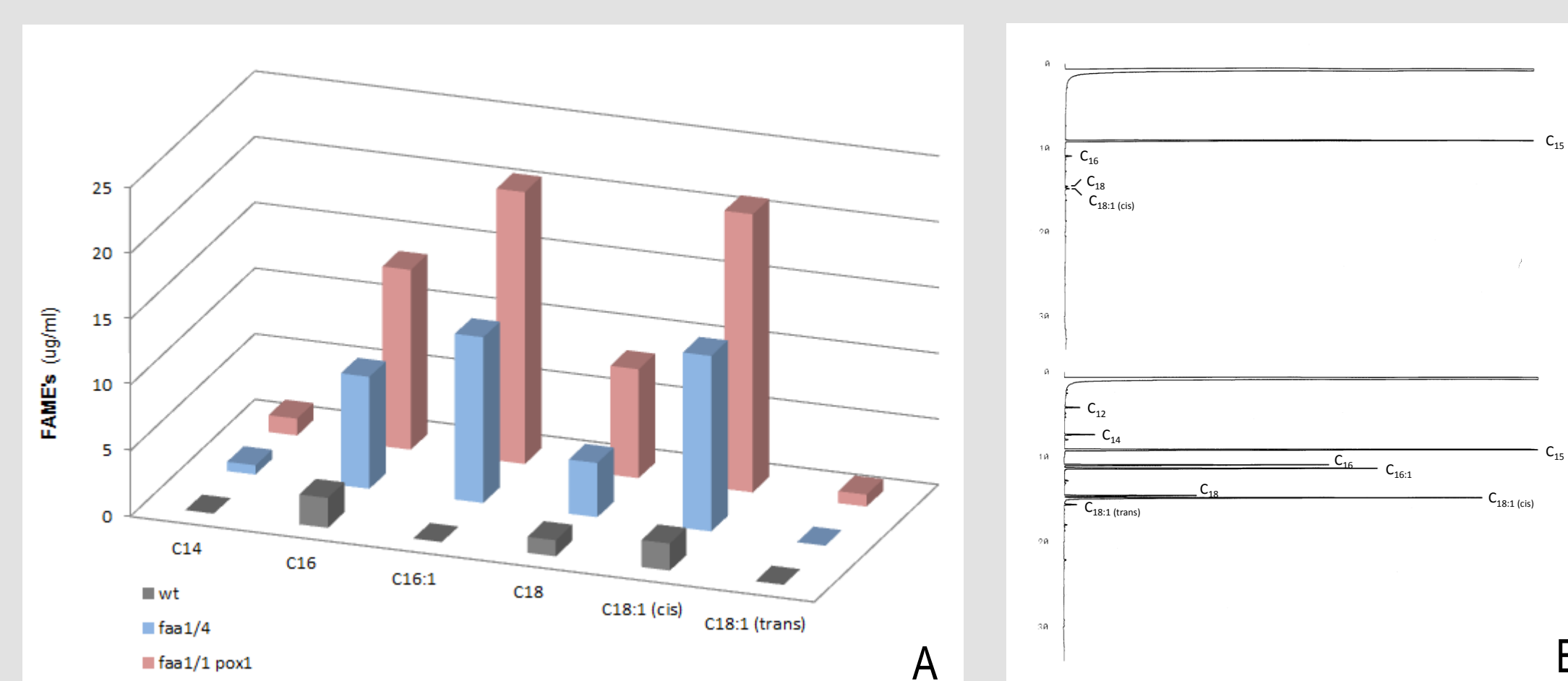


Fig. 3: A) Analysis of the FAMES composition of the culture media, after 72 hours of growth, by GC. B) Chromatographical analysis of the supernatants. up: wild type strain; down: *faa1/4-Δ1 pox1-Δ1* strain.

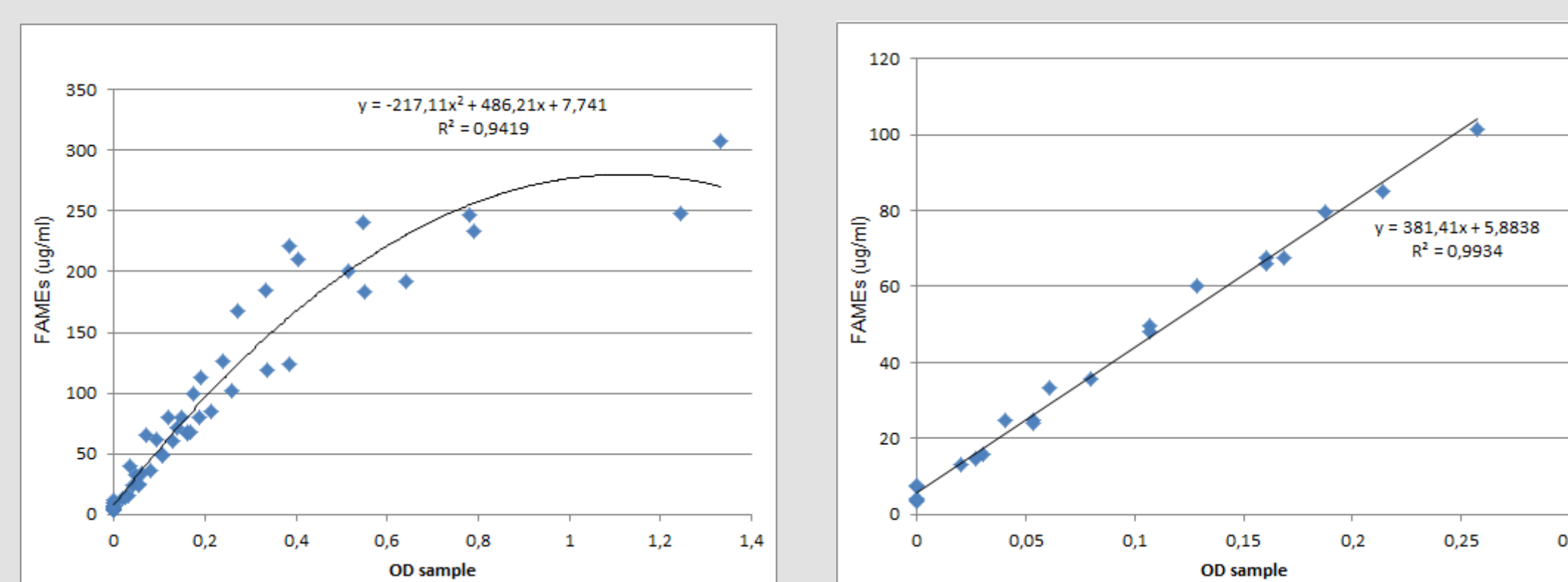


Fig. 4: Correlation between the spectrophotometrical and chromatographic analysis of the supernatants.

Final Remarks

Fatty acids form insoluble particles in culture media of the cells, these can be found as a white precipitate after centrifugation (Fig.1a).

We have found that these particles scatter light in a way that can be measured with a spectrophotometer (Fig.1b). The application of this property as a method for rapid semi-quantitative measurement of fatty acid concentration shows a good correlation between the spectrophotometrical and chromatographic analysis of the supernatants. A linear relationship between $\text{OD}_{600\text{nm}}$ and FAMES concentration was observed when $\text{OD} < 0.25$ (Fig. 4).

The fatty acid production of the modified strains was analysed by optical density of the extracellular medium and gas chromatography and triple knock-out mutant *faa1/4-Δ1 pox1-Δ1* studied in this work shows delayed uptake when compared with the *faa1/4-Δ1* mutant.

References

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Acknowledgments

G. Ribeiro is supported by the FCT grant SFRH/BD/42565/2007.